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(54) Title: METHODS AND COMPOSITIONS FOR SUPPRESSING AN IMMUNE RESPONSE USING MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) CLASS II PEPTIDES

(57) Abstract

Methods and compositions for suppressing an immune response using or comprising peptide fragments of the alpha chain of class II major histocompatibility complex containing a highly conserved motif and effective to suppress at least one and preferably all of the following: a mixed lymphocyte reaction or other T-cell allorecognition reaction; generation of cytotoxic T-cells recognizing an alloantigen; lymphocyte proliferation against tissue antigen; and stimulatory cytokine production by lymphocytes. Immune responses that can be abated or suppressed include alloimmunity and autoimmunity.

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METHODS AND COMPOSITIONS FOR SUPPRESSING AN IMMUNE RESPONSE USING MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) CLASS II PEPTIDES

FIELD OF THE INVENTION

This invention relates to methods and compositions for suppressing various immune responses, including but not limited to proliferation of (host) lymphocytes to: (a) encounter of histocompatibility antigen (i.e. an alloimmune response or response to a xenograft); and (b) antigens present in specific tissues or organs of the body (which includes but is not limited to autoimmune response).

BACKGROUND OF THE INVENTION

The need often arises to suppress, or abate, abnormal or undesirable immune responses. One such situation arises in the context of organ transplantation to prolong transplant survival and to endeavor to avoid transplant rejection (acute or chronic or both). The major histocompatibility complex (MHC) antigens are the principal targets of immune response to allografts. The current accepted hypothesis is that this response is mediated predominantly by a direct pathway, i.e. a direct reaction of host T-cells with intact allo-MHC molecules, although in another pathway (indirect), recognition by host T-cells of allo-MHC peptides fragments presented by self antigen-presenting cells (self-APC), is also involved and appears to play a critical role in allorecognition.

To prolong allograft survival, transplant recipients have heretofore been treated with various immunosuppressant and cytotoxic drugs, all of which are nonspecific (i.e. do not selectively suppress alloimmunity) and eventually cause global immunosuppression, which

increases the susceptibility of the transplant recipient to opportunistic infections and can lead to malignancies. Additionally, several of these drugs have serious or even dangerous side-effects, such as kidney and liver toxicity, as well as hypertension.

Recently, the present inventors and their co-workers have discovered that oral administration of MHC antigens and fragments thereof tolerizes alloimmune responses by inducing tolerance against the "indirect pathway" of alloimmunity which, by an as yet unknown mechanism, also downregulates the "direct pathway", and thereby reduces alloimmune response. These findings have been reported in Sayegh et al., *Transplantation* 57:9, 1994, and are also the subject of U.S. Patent Applications Serial Nos. 07/607,826 and 07/977,737 and their counterpart International Application Nos. PCT/US91/08143 (published as WO 92/07581 on May 14, 1992) and PCT/US93/03708 (published as WO 93/20842 on October 28, 1993).

Others, such as Krensky and co-workers have focused on the use of specific peptides derived from the alpha chain of class I of the MHC to abate alloimmunity. The Krensky peptides are said to inhibit differentiation of cytotoxic T-cells. See, e.g., International Applications: PCT/US94/12985 (published as WO95/13288); PCT/US93/1758 (published as WO93/17699); PCT/US92/9440 (published as WO93/08817); and PCT/US88/0245 (published as WO88/05784). The Krensky peptides are believed to work by binding to T-cells. They inhibit cytotoxic cell function (i.e. preformed cytotoxic T-cells), require the co-presence of cyclosporine to produce an inhibitory effect, and importantly, do not appear to interfere with helper T-cell function. Helper T-cells are responsible for the initiation of all immune responses. The inability to interfere with helper T-cell function is thus a drawback for an agent used to suppress an immune response. The Krensky peptides are likely to be responsible only for a non-specific inhibitory effect.

In addition, Chicz, R.M. et. al. conducted extensive research on Class I and Class II MHC-derived

peptides bound to a Class II MHC molecule HLA-DR1 (Nature, 1992, 358:764). They subsequently reported binding details on a great number of such peptides and hypothesized a physiologic role for them: the authors proposed that nonallele-specific Class II-derived peptides perhaps serve to modulate presentation of foreign antigens (non-MHC) and to broaden the T-cell tolerance to "self" antigens during thymic development. The fact that several of these Class II-derived peptides were quite immunogenic led the authors to postulate that they would play a role in alloreactivity, although they did not say what the role might be. The Chicz et. al. group conducted no studies on inhibitions of immune responses and did not propose any agent or method for decreasing or abating immunity.

Another situation in which an abnormal immune response is in need of suppression is autoimmunity (i.e. the abnormal immune response implicated in autoimmune diseases). In these pathological states, the afflicted individuals mount an immune response to their own antigens, which their immune system mis-recognizes as foreign antigens. In T-cell mediated or T-cell dependent autoimmune diseases in particular, T-cells of the afflicted individuals react with peptides from self antigens presented by self-APC.

The conventional approach for suppressing autoimmune responses has also involved administration of nonspecific immunosuppressant and cytotoxic agents with the same types of disadvantages as described above with respect to alloimmunity.

Recently, co-workers of the present inventors developed a therapeutic regime to induce tolerance in individuals afflicted with autoimmune diseases (especially those that are T-cell mediated or at least have a T-cell component involved in their pathogenesis) by the oral administration of bystander antigens (which are antigens specific to the afflicted tissue and recognized by the T-cells of the afflicted mammal but not necessarily constituting a target of the abnormal autoimmune response). This oral tolerance induction is the subject of the

following U.S. patents and patent applications: U.S. Patent application Ser. No. 08/472,017 (originally filed as 07/596,936 on October 15, 1990 and corresponding to PCT/US91/07542, published as WO92/06708), which generally discloses the treatment of autoimmune diseases by oral administration of autoantigens. U.S. Patent Application Ser. No. 08/472,017 (originally filed as Ser. No. 07/595,468 on October 10, 1990, and corresponding to application PCT/US91/07475 published as WO92/06704) discloses oral and aerosol compositions containing insulin for treating mammals suffering from, or at risk for, Type 1 diabetes. U.S. Patent Application Ser. No. 08/419,505 (originally filed as Ser. No. 07/551,632 on July 10, 1990 and corresponding to PCT/US90/03989, published as WO91/01333) describes treatment or prevention of autoimmune uveoretinitis by, e.g., orally administering S-antigen. U.S. Patent 5,399,347 describes treatment of autoimmune arthritis using e.g., oral type II collagen. U.S. Patent Application Ser. No. 08/419,502 (originally filed as 07/454,486 on December 20, 1989, and corresponding to PCT/US90/07455, published as WO91/08760) discloses aerosol administration of autoantigens. U.S. Patent Application Ser. No. 08/472,017 (originally filed as 07/843,732, on 02/28/92 and corresponding to PCT/US93/01705, published as WO93/16724) describes orally administering "bystander" antigens from the locality of a tissue under autoimmune attack to suppress autoimmune response.

Intensive research in various branches of immunology, including but not limited to research in transplantation and autoimmunity, has revealed that immune responses involve exceedingly complex physiological and biological processes. Accordingly, there is a persistent need in the field for different methods and compositions to modulate immune responses, taking advantage of different characteristics of immune processes.

OBJECTS OF THE INVENTION

One object of this invention is to provide novel compounds and compositions that suppress immune responses,

and methods for using these materials for this purpose.

A more specific object of this invention is to provide compounds and compositions that suppress allorecognition, and therefore abate alloimmunity, and methods for using these materials for this purpose.

Another specific object of this invention is to provide compounds and compositions that suppress an autoimmune response, and therefore abate autoimmunity, and methods for using these materials for this purpose.

Even more specific objects of this invention are to provide

(a) compounds, and compositions that:

(i) inhibit lymphocyte proliferation against allo-MHC; and/or

(ii) inhibit generation of cytotoxic T-cells directed against alloantigen; and/or

(iii) inhibit lymphocyte proliferation against tissue antigen; and/or

(iv) inhibit stimulatory cytokine production by lymphocytes, and

(b) methods for using these compounds and compositions to inhibit one or more of (i) through (iv).

A further object of this invention is to provide compounds and compositions derived from alpha chains of Class II MHC that accomplish one or more of the foregoing and that are not allele-specific (and, optionally, not species-specific) and can be used to modulate one or more of the foregoing immune responses in individuals.

A still further object of this invention is to provide methods, compounds, and compositions down-regulating immune responses, as mentioned above, without causing the serious adverse effects associated with use of conventional global immunosuppressants or cytotoxic agents.

SUMMARY OF THE INVENTION

As one aspect of this invention, we have determined a method for suppressing an immune response that involves using a peptide compound (derived from the alpha

chain of Class II major histocompatibility complex) to suppress such response. The peptide is typically a fragment of that alpha chain and includes a highly conserved region or motif of amino acid residues that is maintained across alleles and species. Other than the highly conserved motif, the peptide can contain one or more portions of the alpha chain adjacent to such motif. In a preferred embodiment, the peptide is composed of 13-26 amino acid residues, and most preferably 16-23 amino acid residues. The highly conserved motif preferably consists of four amino acids and the peptide contains at least three and most preferably all four of these amino acids. Most preferred is the motif consisting of all four of amino acid residues 70-73 of the alpha chain of human DQ Class II major histocompatibility complex (KHNL).

Use of the peptides of the present invention encompasses their delivery to the site of reaction of a T-cell with an APC, e.g. oral, mucosal, or parenteral administration to an individual in need of suppression of an immune response. Such individuals include, without limitation, transplant recipients, prospective transplant recipients, xenograft recipients, and persons afflicted with a T-cell mediated or T-cell dependent autoimmune disease, or determined to be at risk for developing such a disease (by genetic screening or by confirmation of an ongoing autoimmune reaction, such as that involved in the pre-onset stages of juvenile diabetes against pancreatic beta cells).

In another aspect, the invention relates to the foregoing peptides and to compositions containing them suitable for oral or parenteral administration according to the above methods.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A depicts the full amino acid sequences of the first 95 amino acid residues of the alpha chain of the class II MHC from rat RT1.Bα^{l} (top), rat RT1.Bα^{ed} (middle) and a human HLA.DQα 1*0101 (bottom); Figure 1B depicts the same alpha chain fragments as Figure 1A but the homologous

residues from the middle and bottom sequences have been omitted (replaced by hyphens); Figures 1A and 1B highlight the sequence of a peptide within the invention consisting of amino acid residues 62-77 in the bottom sequence and 62-78 in the top sequence (underlined in Figure 1B).

Figure 2 depicts amino acid sequences for full length HLA DQα chains (amino acid residues 1-232) isolated from different human individuals in the same manner as Figure 1B. The stars (*) depict unelucidated residues.

Figure 3 depicts amino acid sequences for HLA DQß chains isolated from different individuals in the same manner as Figure 2.

Figure 4 shows the sequences of examples of peptides within the invention: MHC class II α chain peptides DQα1*0101, RT1.Dα, and RT1.Bα, and the MHC class II ß chain peptide DQß 0501.

Figures 5 and 6 are tables that show the percent inhibition exhibited in one way rat mixed lymphocyte response (MLR) experiments ("LEWxWF", "LEWxBN". "BNxLEW", and "BNxWF"), EAE experiments ("Antigen/MBP"), mitogen proliferation experiments ("ConA"), and human MLR experiments ("Human") using the DQα1*0101 ("DQα") peptide. Negative values denote augmentation of immune response.

Figure 7 is a table showing the percent inhibition of various immune responses exhibited in various experiments using the RT1.Dα peptide.

Figures 8 and 9 are tables similar to Fig. 7 showing the percent inhibition exhibited in experiments using the RT1.Bα peptide.

Figures 10 and 11 are tables similar to Fig. 7 showing the percent inhibition exhibited in experiments using the DQß peptide.

Figure 12 is a graph of the percent inhibition of rat (LEWxWF) MLR accomplished by various doses of DQα*0101 peptide and DQβ*0501 peptide.

Figure 13 is the same type of graph as Figure 12 for human MLR.

Figure 14 is a graph of the percent specific lysis

of WF blast cells by LEW effector cells generated in the presence of DQα peptide.

Figures 15 and 16 are graphs of γ-interferon (Fig. 15) or interleukin-2 (Fig. 16) concentrations in culture supernatants (human MLR) on day 3 in the presence of various peptides (DQα or DQβ) at different concentrations, or in the absence of peptide.

DETAILED DESCRIPTION OF THE INVENTION

The contents of all patent applications, patents and literature references referred to in this specification are hereby incorporated by reference in their entirety. In case of inconsistency, the present disclosure (including its definitions) will control.

The major histocompatibility complex (MHC) plays a central role in many aspects of immunity. In alloimmunity, histocompatibility (a/k/a transplantation) antigens of a donor react directly with host T-cells. In ordinary immune response against foreign antigens or antigens involved in autoimmunity, host MHC presents peptide fragments of these antigens to host T-cells only.

There are two types of MHC protein molecules: Class I and Class II. Class I MHC proteins are present on virtually all tissues. Class II MHC proteins are present on the surface of only certain immune system cells. The human MHC genes (i.e. the HLA genetic locus) are located on human chromosome 6, while the mouse MHC genes are located in the H-2 genetic locus on mouse chromosome 17. The corresponding rat MHC genes are referred to as "RT1".

Class II MHC molecules are membrane glycoproteins that form part of the MHC and are most important in the initiation of immune responses. They are found mainly on cells of the immune system, including activated T-cells, B-cells, macrophages, brain astrocytes, epidermal Langerhans cells, dendritic cells, and thymic epithelium. Class II MHC molecules play a central role in immune response during tissue graft rejection, graft-versus-host reactions, stimulation of antibody production, and in the recognition

of "self" (or autologous) antigens, among other immune events.

CD4$^+$ T-cells (helper T-cells) recognize antigen only when it is presented in connection with Class II MHC of antigen-presenting cells (hence the term "Class II - MHC restricted"). In turn, these cells are the initiators of any immune response. For this reason, it is most efficient to target CD4$^+$ T-cells in any attempt to suppress an immune response.

According to the present invention, one or more peptides of the invention are placed in contact with reacting immune system components (e.g. T-cells and APC's) to suppress an immune response in need of suppression. The peptides are derived from (i.e. are fragments of) the alpha chain of the class II MHC and comprise at least one highly conserved motif of at least 3, and preferably of 4, consecutive amino acids.

In the following discussions the following terms shall have the meaning ascribed to them below.

"Oral administration" shall mean both oral administration and enteral administration (delivery directly into the stomach).

"Mucosal administration" shall mean administration by delivery to the buccal, nasal, bronchial or pulmonary mucosa.

"Parenteral administration" means administration by the intravenous, subcutaneous, or intramuscular route.

The present inventors found that the following peptide from the alpha chain of Class II MHC: RT1.Dα^u (51-75): FASFEAQGALANIAVDKANLDIMIK inhibited alloimmune response in vitro (MLR). Specifically, the foregoing rat peptide (51-75) inhibited LEW x WF MLR in a dose-dependent fashion (75 $\pm$ 7% inhibition with 500 μg/mL, n=9). The rat peptide 51-75 also achieved considerable inhibition (60 $\pm$ 12% at 500 μg/mL) of the MLR of LEW x BN, showing that the inhibitory effect was not strain-specific. Additionally, the rat peptide 51-75 inhibited the human MLR (using 2 x 10^5 responder peripheral blood mononuclear cells and an equal

amount of allogeneic irradiated stimulator cells in a standard one-way human MLR as described in Eckels, DD. et al. PNAS (USA), 1988, 85:8191) to an extent of 72 ± 5% with 500 μg/mL, n=11. This showed that the inhibitory activity of this peptide was not species specific. A comparison with the corresponding sequence of the human HLA-DR alpha chain revealed identity except at two residues: the human sequence had E (instead of L) as the 71st residue, and T (instead of I) as the 74th residue.

Rat peptide 51-75 also inhibited (100% inhibition with 500 μg/mL of peptide) generation of cytotoxic T-cells (CTL) but not the function of preformed cytotoxic T-cells. The same peptide did not inhibit lymphocyte proliferation to mitogen (in the absence of stimulator cells), indicating that the MLR inhibition was directed at the interaction between the T-cell receptor of the responder cells and the MHC of the stimulator cells and not to a nonspecific toxic or inhibitory effect on T-cells. The foregoing results with rat peptide 51-75 have been published by the present inventors in *Transplantation Proceedings*, 1995 (February issue) 27:409.

Another rat peptide from the same alpha chain composed of residues 26-50 having the sequence FDGDEIFHVDIKKSETIWRLEEFAK failed to inhibit any of the foregoing MLR's or to inhibit CTL generation.

The present inventors also tested various other peptides from the human HLA DQα chain and from other rat alpha chain.

These peptides had the following sequences:

DQα1 0101	(62-77):	ALRNMAVAKHNLNIMI
RT1.Dα	(61-75):	ANIAVDKANLDIMIK
RT1.Bα	(62-78):	GLQNIAIIKHNLEILMK

Human peptide DQα1*0101 (62-77) inhibited the same immune responses as rat peptide 51-75 but did so completely at exquisitely low concentrations (as low as 5 μg/mL and up to 100 μg/mL) as described in the following examples. Like that of RT1.Dα^{u} (51-75), the inhibitory effect of DQα1*0101 (62-77) was not due to lymphocyte toxicity, nonspecific

inhibition or interference with the function of preformed CTL.

The superior inhibitory effects of the human peptide DQ∝1*0101 (62-77) extended to rat MLR demonstrating the nonspecies specificity of the human peptide (LEW x WF, LEW x BN and BN x WF).

The two peptides from alpha chain of Class II MHC of rat RT1.D∝ (61-75) and RT1.B∝ (62-78) also inhibited the rat MLR reactions LEW x WF, LEW x BN and BN x WF but were less effective in inhibiting the human MLR. In addition, peptide RT1.D∝ (61-75) was less effective than peptide RT1.B∝ (62-78).

Another peptide from the beta chain of human HLA, peptide:

DQβ 1 0501 (62-77): NSQKEVLEGARASVDR

failed to inhibit all of the MLR and CTL reactions described above, and was used as a negative control.

The alpha chain peptides that were effective possess substantial homology not only between alleles but also between species. Examination of their structural differences correlated with inhibitory activity as follows.

The alpha peptides that had the highly conserved 4-amino acid motif K-NL had substantial inhibitory activity while those that did not had no inhibitory activity (the beta-chain human peptide did not possess this motif, either). The peptides where the motif was KANL were less active than the peptides where the motif was KHNL. That this motif is highly conserved among species and human alleles is demonstrated in Figures 1 and 2.

Because of its high inhibitory activity, the human peptide DQ∝1*0101 (61-77) is preferred for use in inhibiting transplantation immunity (allograft rejection).

Those of the foregoing peptides that inhibited MLR also inhibited proliferation of T-cells that recognized MBP taken from rats in which EAE was induced by immunization with myelin basic protein. Again, the human peptide DQ∝1*0101 (62-77) was a potent inhibitor, although in the context of autoimmunity in this rat model, the peptide

RT1.Bα (62-78) appeared to be significantly more potent.

Inhibition of immune response in the autoimmune model demonstrates that the inhibitory activity of the peptides of the invention extends beyond alloimmunity to ("ordinary") immunity against foreign antigen and to autoimmunity.

Peptides for use in the present invention can be synthesized using well known solid phase synthesis techniques (such as those of Merrifield, R.B. Fed. Proc. Am. Soc. Ex. Biol., 21: 412, 1962 and J. Am. Chem. Soc. 85: 2149, 1963; Mitchel, A.R- et al., J. Am. Chem. Soc. 98: 7357, 1976; Tam, J. et al., J. Am. Chem. Soc. 105: 6442, 1983), preferably in commercially available automated peptide synthesizing equipment. They can also be synthesized using recombinant DNA technology that is well known in the art. Aside from the sequences disclosed herein, amino acid sequences of Class II MHC alpha chain antigens are available from the Gene Bank database. They have also been published in various references and are known in the art.

The peptide selected for immunosuppression has a highly conserved region (or "motif"), not necessarily the K-NL motif. In this sense, "highly conserved" means that the motif is conserved among several alleles and preferably across species as well. The peptide is derived from the alpha chain of the class II MHC. Although the peptide is preferably 13-26 amino acids in length, it is understood that longer peptides that are to be exposed to degradation, such as through the gastrointestinal tract, can present a smaller peptide of this length. Thus, an orally administered peptide could be longer, and could be naturally processed in the body to reach the preferred length. The active peptide can also be in conjugated form, i.e. conjugated to other peptide sequences or carriers.

A peptide selected according to the above criteria can be tested for operability by experimentally determining whether it is suppressive in a T-cell allo-proliferation assay (e.g., an MLR), is suppressive in an antigen specific

T-cell proliferation assay, suppresses the generation of cytotoxic T-cells that recognize an alloantigen, or suppresses (e.g., in MLR) the production of stimulatory cytokines, particularly IFN-gamma (gamma interferon) or IL-2 (interleukin 2). Experimental protocols for determining such suppression are described in detail in the Examples below.

Peptides for screening in the invention include, for example, those that are 13-26 amino acids long, and contain a sequence shown in Figure 2 that contains the tetrapeptide KHNL. Examples of such peptides include FDPQGALRNMAVAKHNLNIM, TNIAVLKHNLNILIKRS and KHNLNSLIKRSNSTAATN.

When used to suppress an alloimmune response (graft rejection, graft v. host disease or xenograft rejection), the peptides can be administered to individuals who are to receive an allograft, such as an organ transplant, or who have already received an allograft. It is believed that to be most effective, the peptides of the present invention should be administered no later than 7 days after transplant, and preferably less than one day. A peptide is preferably first administered between about 7 and about 14 days before the transplantation procedure. The treatment is preferably continued longer than about 6 months after e.g., an organ or tissue has been transplanted into the individual.

When it is desired to use the peptides to suppress antigen-specific response (e.g. in autoimmunity), they may be administered either prior to the onset of symptoms of autoimmune disease, or after such symptoms have appeared. In the case of Type I diabetes, for example, the peptides might be administered to an individual found to exhibit an autoimmune reaction against pancreatic ß cells (or at high risk for developing such a reaction) but not yet exhibiting any hyperglycemia or hypoinsulinemia. Suppression of autoimmune response is preferably carried out before the substantial destruction of tissues that the response is directed against. Other autoimmune diseases in which

treatment with the present peptides is useful include without limitation multiple sclerosis (for which EAE is a model), rheumatoid arthritis (for which collagen-induced arthritis is a model), uveoretinitis (for which EAU, i.e. experimental autoimmune uveoretinitis, is a model), autoimmune thyroiditis, systemic lupus erythematosus, myasthenia gravis, glomerulonephritis, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, pemphigus vulgaris, Grave's disease, mixed connective tissue disease, ulcerative colitis, primary biliary cirrhosis, myositis, Wegener's granulomatosis, male infertility (sperm antigen immunity), Type II-diabetes and more generally insulin resistance and pernicious anemia.

When a peptide according to the invention is to be ingested by the individual, via the oral or enteral route, it is preferably administered in an amount of between about 100 μg/kg per kg of body weight and about 200 mg per kg of body weight per day. It may be administered as a single dose or in multiple doses daily. Preferably, it is administered in an amount between about 1 mg and about 50 mg, and most preferably between about 10mg and about 50mg, per kg body weight of the individual per day. The exact amount to be administered can vary depending on the specific activity of a particular peptide, as well as the severity and stage of a patient's disease and/or the physical condition of the patient, and is subject to optimization as is well-known in the art.

The oral pharmaceutical formulations of the present invention may contain inert constituents including pharmaceutically acceptable carriers, diluents, fillers, solubilizing or emulsifying agents and salts of the type that are well-known in the art. For example, tablets and caplets may be formulated in accordance with conventional procedures employing solid carriers, such as starch and bentonite, that are well-known in the art. Examples of solid carriers include bentonite, silica, dextrose and other commonly used carriers. Further non-limiting examples of carriers and diluents which may be used in the formulations

of the present invention include saline and any physiologically buffered saline solution such as phosphate buffered saline, pH 7-8 and water.

Capsules containing the peptide may be made from any pharmaceutically acceptable material, e.g, gelatin or a cellulose derivative. The peptide may be administered in the form of sustained release oral delivery systems and/or enteric coated oral dosage forms, such as is described in U.S. Patent No. 4,704,292 issued November 3, 1987, U.S. Patent No. 4,309,404 issued January 5, 1982, or U.S. Patent No. 4,309,406 issued January 5, 1982.

The amount of peptide contained in an individual dose need not in itself constitute an effective amount for suppressing immune response, since the necessary effective amount can be reached by administration of more than one dose.

For the purpose of parenteral administration, the active compounds of the invention can be incorporated into a physiologically acceptable solution or suspension. These preparations preferably contain from about 100 μg of active compound per kg to about 200 mg per kg of body weight thereof. Preferred compositions and preparations according to the present inventions are prepared so that a parenteral dosage unit contains from about 1 mg to about 50 mg of active compound per kg body weight. Most preferred is from about 10 mg to about 50 mg active compound per kg of body weight.

The solutions or suspensions can also include the following components: a sterile diluent such as, for example, water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol, other synthetic solvents, and the like; antibacterial agents such as, for example, benzyl alcohol, methyl parabens, and the like; antioxidants such as, for example, ascorbic acid, sodium bisulfite, and the like; chelating agents such as, for example, ethylenediamine tetraacetic acid and the like; buffers such as, for example, acetates; citrates, phosphates, and the like, and agents for the adjustment of

toxicity such as, for example, sodium chloride, dextrose, and the like.

The parenteral multiple dose vials can be of glass or plastic materials.

In mucosal administration, the active protein is placed in contact with the buccal, nasal, bronchial or pulmonary mucosa. Formulations useful for mucosal administration include those suitable for administration of polypeptides across the mucosal membrane. For example, U.S. Patent Nos. 4,226,848 and 4,690,683 describe polymeric matrices useful in administering pharmaceuticals into the nasal cavity. U.S. Patent No. 4.952,560 discloses an ointment formulation comprising a water-soluble protein and a monohydric alcohol which may be suitable for use in administering the present invention because it increases absorption of drugs across epithelial barriers. Methods of improving transcutaneous absorption of materials is described in U.S. Patent No. 4,272,516. Each of these formulations and others well known in the art may be used for mucosal delivery of bystander antigen as described in the present invention.

Additional suitable formulations include commercially available vehicles and formulations which may but need not include surface active agents and other skin penetrants as absorption promoters. Specifically, U.S. Patent No. 5,407, 911 describes the use of axacycloalkane derivatives as absorption promoters for high molecular weight polypeptides. U.S. Patent No. 5,397,771 describes the use of n-glycofurols in methods of administering pharmaceutical compositions across the mucosal membrane. Additionally, U.S. Patent No. 4,548,922 discloses the use of water-soluble amphophilic steroids to increase absorption. Gel-based compositions, such as those described in Morimoto et al. (*Chem. Pharm. Bull. 35*(7):3041-3044) are also suitable for the present invention.

Where the peptide is administered mucosally, the quantity of peptide administered in, e.g., an aerosol dosage form by inhalation, is preferably between about 100 μg and

200 mg per kg body weight per day, preferably between 1-50 mg/kg of body weight, and most preferably between 10-50 mg/kg of body weight. The by-inhalation forms of the present invention may be administered to a patient in a single dosage form or multiple dosage forms. The exact amount to be administered may vary depending on the state and severity of any disease to be treated, the activity of the patient's immune system and the physical condition of the patient.

Inhalable aerosol or spray pharmaceutical formulations may include, as optional ingredients, pharmaceutically acceptable carriers, diluents, solubilizing or emulsifying agents, and salts of the type that are well-known in the art. Specific non-limiting examples of the carriers and/or diluents that are useful in the aerosol pharmaceutical formulations of the present invention include water, normal saline and physiologically-acceptable buffered saline solutions such as phosphate buffered saline solutions, pH 7.0-8.0.

Examples of useful solubilizing and emulsifying agents are physiologically balanced salt solutions, phosphate buffered saline and isotonic saline. The salts that may be employed in preparing mucosal dosage forms of the invention include the pharmaceutically acceptable salts of sodium and potassium.

Aerosol compositions can be administered, e.g., as a dry powder or preferably in an aqueous solution. Preferred aerosol pharmaceutical formulations may comprise, for example, a physiologically-acceptable buffered saline solution containing between about 7 mg and about 700 mg of the peptide of the present invention.

Dry aerosol in the form of finely divided solid particles that are not dissolved or suspended in a liquid are also useful in the practice of the present invention. The compositions used in the present invention may be in the form of dusting powders and comprise finely divided particles having an average particle size of between about 1 and 5 microns, preferably between 2 and 3 microns. Finely

divided particles may be prepared by pulverization and screen filtration using conventional techniques that are well known to those skilled in the art. The particles may be administered by inhaling a predetermined quantity of the finely divided material, which can be in the form of a dry atomized powder.

The invention can also be used to suppress immune response in veterinary medicine, animals used in laboratory research, and in suitable human patients.

The present invention is illustrated by the following examples, which are intended to illustrate the invention without limiting its scope.

Example 1: Inhibition of Rat Mixed Lymphocyte Response (MLR) By Peptides From the Class II MHC Alpha Chain

Experiments were conducted that demonstrated that three peptides (two from rat and one from human) from the alpha chain of the class II MHC inhibited rat MLRs. A peptide from the beta chain did not inhibit the rat MLRs. The four peptides tested are shown in Figure 4. They were derived from the alpha chain of the class II MHC. Two were derived from human class II MHC: DQα1*0101 (amino acids 62-77) and DQß1*0501 (amino acids 62-77), and two were derived from rat class II MHC: RT1.Dα (amino acids 62-78) and RT1.Bα (amino acids 62-77).

Lymph nodes were taken from LEW (typically used as responders) and WF or BN rats (typically used as stimulators). Rats of the Lewis (LEW), Wistar Furth (WF) and Brown Norway (BN) variety were used. The excised nodes were then pressed through stainless steel mesh and suspended in medium containing RPMI with L-glutamine, 10% fetal calf serum, 1M HEPES, penicillin and streptomycin, and 5 $\times 10^{-5}$ M of 2-ME (mercaptoethanol). The lymph node cells were then washed twice and resuspended in the same medium at 3 $\times$ 10^6 cells/mL. Stimulator cells were irradiated (3000 Rads). 100 μl (3 $\times$ 10^5 cells) each of responders and stimulators were seeded onto a 96-well flat-bottomed culture plate. Serial dilutions (0-10μg/mL) of the peptides shown in Figure

4 were added into separate wells. The plate was incubated at 37°C for four days before being pulsed for 6 hours with ^3H thymidine (1 microCi/well). Cell proliferation was monitored by incorporation of the ^3H thymidine as measured using a liquid scintillation counter.

Results of the rat MLR experiments are shown in Figures 5, 7, 8 and 10 for different combinations of rat responder and stimulator cells. In Figure 5, for example, "LEWxWF" results are shown for the human DQα peptide (i.e. the "DQα1 0101" peptide shown in Figure 4). "LEW" is the responder cell, while "WF" is the stimulator cell. Results of three experiments for this (responder)x(stimulator) combination are shown. Results are also shown for the combinations "LEWxBN", "BNxLEW", "BNxWF" and "LEWxBN". 100% inhibition of the MLR for the DQα peptide was achieved at 10μg mL in the LEWxWF and BNxWF MLRs. This was a very high inhibitory activity. 100% inhibition was achieved using 100 μg/mL in the LEWxBN system. Thus, the inhibition using the DQα peptide was strong and was not strain specific.

As shown in Figures 7 and 8, the rat peptides derived from the alpha chain of the class II MHC, RT1.Dα and RT1Bα also inhibited rat MLRs. The RT1.Bα peptide achieved 100% inhibition in the LEWxWF and BNxWF MLRs at 10 μg/mL. The RT1.Dα peptide was somewhat less inhibitory. It achieved 100% inhibition of the BNxWF MLR at 100 μg/mL but was less inhibitory in the other rat MLRs.

Figure 10 shows the results obtained in rat MLRs using the DQß (i.e. the "DQß1 0501" peptide). No inhibition occurred.

Results of the LEWxWF MLR for the DQα and DQß peptides are graphically shown in Figure 12. The graph depicts the complete inhibition found with a dose of 10μg/mL of the DQα peptide and the lack of inhibition with the DQß peptide.

Example 2: Inhibition of Human MLR By Peptides From the Class II MHC Alpha Chain

Human peripheral blood mononuclear cells (PBMC) for use in the human MLR were prepared as follows. 20 mL of blood was placed in a 50 mL Nunc™ tube and diluted to 32 mL with medium containing RPMI, penicillin/streptomycin and HEPES. This was underlayed with 12 mL of Ficoll™ and spun in a centrifuge at 2000 RPM for 30 minutes. An interface layer formed of a "Buffy coat" of cells was removed, and those cells placed in another 50 mL Nunc™ tube. These tubes were filled with 50 mL of the medium described above and spun at 1500 RPM for 10 minutes. Liquid was then removed from the tubes and the pellet resuspended in 5 mL of the same medium, combining cells from 2 separate tubes. Medium was added to arrive at 50 mL. The suspension was then spun at 1200 RPM for 10 minutes. Liquid was removed and the pellet, resuspended in 4 mL of 10% medium (the above described serum in medium at a ratio of 1:10) and the cells counted. Further 10% medium was added to arrive at a concentration of 4 x 10^6 cells/ml.

A human MLR was performed as follows. Stimulator PBMC were irradiated at 3000 rads. 100 μL of the 10% medium described above was added to each well of a 96 well U-bottomed plate. Experimental groups were set up in quadruplicate. In the first row of each plate, to which the highest concentration of peptide was to be added, a further 60 μL of medium was added. To four of these wells, 40 μl of DQα peptide was added at a concentration of 1 mg.ml. In the adjacent four wells, 40 μl of DQß peptide was added at a concentration of 1 mg/ml. The contents of each well was mixed with a multichannel pipette. 100 μl was removed from each well, and mixed with 100 μl of media in each well of the next row of the plate. This was repeated for each row in the plate. 50 μl of irradiated stimulator cells (2 x 10^6 cells) was then added to each well, except for the control wells (which contained only media). 50 μl of responder cells (2 x 10^6 cells) were then added to all wells, including the control wells. The plates were incubated at 37°C. On

the evening of the 5th day following the start of incubation, each plate was pulsed with ^3H thymidine. 18 hours later the plates were harvested.

Different combinations of human stimulator/responder cells were tested for each peptide. The results obtained with the DQα peptide are shown in Figure 6 ("Human"). The results for "MxC" are those obtained using responder PBMC from individual "M" and stimulator PBMC from individual "C". 100% inhibition was found at 100 μg/mL.

Similar human MLRs were performed using the other (rat) peptides listed in Figure 4, with somewhat lower amounts of inhibition resulting, as shown in Figures 7 and 9.

Figure 13 is a graph of the combined results showing percent inhibition of human MLR using the DQα and DQß peptides.
100% inhibition is indicated using 100 μg of the DQα peptide. Thus, the DQα peptide was not species specific since it strongly inhibited both the rat and the human MLR. The DQß peptide was ineffective.

Example 3: Inhibition of Cytotoxic T-cell (CTL) Generation

The effect of the DQα and DQß peptides on cytotoxic T-cell generation was tested. Equal numbers of responder LEW and irradiated stimulator WF cervical lymph node cells were incubated for 7 days at 37°C at a concentration of 1 x 10^6 cells/mL in bulk cultures, with varying amounts of the peptides being added to the cultures. Primed LEW effector cells from these LEWxWF bulk cultures were tested for their ability to lyse ^{51}Cr-labeled target WF blasts (formed by 2-day incubation with 10 μg/mL ConA) in a 4-hour cytotoxic assay. Sayegh et al., *Transplantation* 51:281, 1991.

The results from this experiment are graphically shown in Figure 14 expressed as percent lysis of target-blasts (indicated by the amount of ^{51}Cr released in the various cultures (over spontaneous release) and compared to

the amount (over spontaneous release) of ^{51}Cr for maximum lysis of a control cultured in detergent obtained at 50 μL target preparation and 150 μL isoterge detergent). Lysis was measured at an effector/target ratio of 50:1 and 100:1. The DQα but not the DQß peptide was found to inhibit cytotoxic T-cell generation in a dose-dependent fashion. Maximum inhibition (5% lysis or less) was obtained with 5μg or 10μg DQα peptide.

The same results are obtained (qualitatively) with rat peptides. (Results not shown).

Example 4: Effect of Peptides on Preformed Effector Cytotoxic T-Cells

As part of an effort to exclude the possibility that CTL inhibition might be due to a toxic effect by the peptides on T-cells (or possibly another nonspecific effect), the effect of the DQα and DQß peptides on preformed effector cytotoxic T-cells was tested. Preformed LEW effector cytotoxic T-cells were generated from LEWxWF bulk cultures as in Example 3, except that no peptides were present in the bulk cultures. Harvested LEW effector cells were then pre-incubated with 10 μg/mL of peptides for 1 hour. The effector cells were then tested for their ability to lyse ^{51}Cr labelled WF target blasts in a 4-hour cytotoxic assay as in Example 3. Neither peptide was found to inhibit the preformed effector cytotoxic T-cells at 10 μg/ml.

The results for effector:target ratios of 100:1 and 50:1 expressed as % lysis (as in Fig. 14) are shown in Table I below:

Preformed Cell Mediated Lympholysis (CML)

TABLE I

	200:1	100:1	50:1	25:1
No Peptide		24	22	18
DQα 10μg/ml		24		

These results illustrate the selectivity of DQα

for $CD4^+$ T-cells and not $CD8^+$ effector cells.

Example 5: Effect on Proliferation to Mitogen

To further exclude the possibility of a nonspecific T-cell suppression or toxicity, the response of LEW cells to the mitogen ConA (in the absence of stimulator cells] was examined. Responder LEW lymph node cells were cultured in 96 well flat bottom microtiter plates with ConA at a concentration of 10 μg/mL and various doses of DQα. Controls were run without ConA and without the peptides. Results shown in Figure 6 ("ConA") do not indicate significant inhibition of proliferative responses to mitogen.

This result confirms that the inhibitory effect of the present peptides is neither toxic nor nonspecific.

Example 6: Effect of Peptides on Cytokines

The effects of the DQα and DQß peptides on upregulatory cytokine production in the human MLR was measured by ELISA on each day for seven days of incubation using supernatants from cell cultures and ENDOGEN kits for human cytokines. Both dose-response and time course experiments were performed. The dose-response results, illustrated in Figures 15 and 16 for day 3, demonstrate complete suppression of each of IFN-γ and IL-2 by the DQα peptide but not by the DQß peptide. (The presence of these cytokines is associated with upregulation of immune responses.) In the time-course experiment (data not shown) 100 μg/mL of peptide was used in the cultures. Supernatants were collected each day from quadruplicate cultures for seven days and assayed at the end of that period. The human DQα peptide at this concentration effectively inhibited both IL-2 and IFN-γ over the seven-day period.

Example 7: Effect of Peptides on Autoimmune Reaction

The effect of peptides according to the invention on the proliferation of rat lymphocytes in the experimental allergic encephalomyelitis (EAE) model of multiple sclerosis

was studied. EAE can readily be induced in small mammals by immunization with myelin basic protein (MBP) in an appropriate adjuvant or by adoptive transfer through the injection of $CD4^+$, MBP-reactive T-cells (Alvord Jr, E.C., et al. eds. in Experimental Allergic Encephalomyelitis: A Useful Model for Multiple Sclerosis, A. R. Liss, N.Y., 1984; Makhtarian, D.E., et al. Nature 305:356, 1984; Ben-Nun, A. et al. J. Immunol. 129:303, 1982). The T-cells that induce EAE in both mice and rats, termed encephalitogenic cells, specifically recognize immunodominant regions of MBP. Experiments were carried out to determine if the peptides of the alpha chain MHC could inhibit T-cell proliferation in the EAE model.

LEW rats were immunized subcutaneously in the footpad with 100 μl of a mixture of MBP and Complete Freund's Adjuvant (CFA). The mixture contained 1 mg/mL of MBP and 4 mg/mL of CFA in a 1:1 volume ratio. Seven days after immunization, popliteal lymph notes from the rats were harvested. The lymph nodes were pressed through stainless steel mesh and suspended in medium containing RPMI with L-glutamine, 10% fetal calf serum, 1M Hepes, penicillin and streptomycin, and 5 $\times 10^{-5}$ M of 2-ME (2-mercaptoethanol). The lymphocytes were washed twice, the cells counted, and then resuspended in 10% FCS at a cell concentration of 2 x 10^6/mL.

Flat bottom microtiter plates were set up containing half dilutions of the DQα, DQß, RT1.Bα and RT1.Dα peptides. 10 μl (50 μg/mL) of MBP was added to each well. 2 x 10^5 lymphocytes were also added to each well. Two controls were used: one contained medium and cells and the other contained media, cells, and MBP. The plates were incubated at 37°C for 4 days. The plates were pulsed with ^{3}H thymidine, harvested after 6 hrs, and measured in a scintillation counter.

Results from these experiments are shown in Figures 5, 7, 8, and 10 ("Antigen/MBP"). The results show that the α chain derived peptides caused 100% inhibition of lymphocyte proliferation at 100 μg/mL, while the ß chain peptide was not effective at preventing proliferation at

even 250 μg/mL.

Example 8: Additional Peptides from the class II MHC α chain

Two additional peptides derived from the non-polymorphic RT1.D^u (DR or IE like) alpha chain of the rat class II MHC were also tested as described above in Examples 1-4. Peptide 1 consisted of residues 26 to 50 and peptide 2 consisted of residues 51 to 75. Peptide 2 had the following sequence:

FASFEAQGALANIAVDKANLDIMIK

Peptide 2 was found to inhibit the LEWxWF MLR at about 75% inhibition with 500 μg/mL. It also inhibited LEWxBN MLR at about 60% inhibition with 500 μg/mL, and human MLR at about 72% inhibition with 500 μg/mL. Thus, MLR inhibition by peptide 2 was neither strain nor species specific. Although Peptide 2 achieved substantial inhibition, it did so at concentrations 5- to 50-fold higher than the human DQα, rat RT1.Dα and rat RT1.Bα peptides tested in Examples 1 and 2. Peptide 1 was not inhibitory in these systems.

Peptide 2 inhibited generation of cytotoxic lymphocytes in a dose-response fashion (100% inhibition with 500 μg/mL of peptide). Incubation of preformed effector cytotoxic T-cells with peptide 2 prior to addition of targets failed to inhibit lysis.

WE CLAIM:

1. A method of suppressing an immune response comprising administering an class II major histocompatibility complex alpha chain, or a fragment thereof that is effective to suppress a mixed lymphocyte reaction to an individual in need of such suppression.

2. The method of claim 1 wherein said individual is the recipient or prospective recipient of an allograft, and said immune response is allograft rejection.

3. A method of suppressing immune response comprising administering a peptide to an individual in need of such suppression, said peptide being a fragment of Class II MHC alpha chain containing at least three of amino acids 70-73 of the class II major histocompatibility complex alpha chain, wherein said peptide is effective to suppress a mixed lymphocyte reaction.

4. The method of claim 3 wherein said peptide contains amino acids 70-73.

5. The method of claim 3 wherein said peptide contains the sequence KHNL.

6. The method of claim 3 wherein said peptide is composed of at most about 23 amino acids residues.

7. The method of claim 3 wherein said peptide has the sequence of the entire class II major histocompatibility complex alpha chain.

8. The method of claim 3 wherein said peptide has a length of between about 16-23 amino acids.

9. The method of claim 3 wherein said individual is the recipient of an allograft.

10. The method of claim 7 wherein said peptide has a sequence that is selected from the group consisting of

(A) a sequence of Figure 2;

(B) an alpha sequence of Figure 1;

(C) a fragment of a sequence of (A) or (B)

that is effective to suppress a mixed lymphocyte reaction.

11. The method of claim 3 wherein said peptide

has the sequence ALRNMAVAKHNLNIMI.

12. The method of claim 3 wherein said peptide has the sequence GLQNIAIIKHNLEILMK.

13. The method of claim 3 wherein said peptide has the sequence ANIAVDKANLDIMIK.

14. The method of claim 3 wherein said peptide is orally administered.

15. The method of claim 3 wherein said peptide is parenterally administered.

16. A peptide of about 23 amino acids in length having the sequence of a fragment of the class II major histocompatibility complex alpha chain including amino acids 70-73, wherein said peptide is effective to suppress a mixed lymphocyte reaction.

17. The peptide of claim 16 which is greater than about 10 amino acids in length.

18. The peptide of claim 16 which is greater than about 16 amino acids in length.

19. The peptide of claim 16 extending from about amino acid 62 to about amino acid 78.

20. A pharmaceutical composition comprising the peptide of claim 16 in combination with a pharmaceutically acceptable carrier.

21. A pharmaceutical composition comprising a class II major histocompatibility complex alpha chain, or a fragment thereof that is effective to suppress a mixed lymphocyte reaction, in an amount effective to suppress an immune response upon contacting a T-cell reacting with an antigen-presenting cell.

22. A method of suppressing an autoimmune response comprising administering an class II major histocompatibility complex alpha chain, or a fragment thereof that is effective to cause suppression in an antigen specific T-cell assay to an individual in need of such suppression.

23. A method of suppressing a response selected from the group consisting of antigen specific T-cell proliferation, generation of cytotoxic T-cells directed

against allogen and lymphocyte proliferation against allo-MHC comprising exposing said antigen specific T-cells, cytotoxic T-cells or lymphocytes proliferating against allo-MHC to a peptide of a class II major histocompatibility complex alpha chain containing a highly conserved region.

24. The method of claim 23 wherein said response is T-cell proliferation against myelin basic protein.

FIG. 1A

```
                               1        10        20        30
                               .         .         .         .
MPLSRALILGVLALTTMLSPCGGQDDIEADHVAAYGINMYQYYESRGQFTHEFDGDEEFYVD
                                    DHVGSYGITVYQYHESKGQYTHEFDGDERFYVD
                               EDIVADHVASCGVNLYQFYGPSGQYTHEFDGDEEFYVD
```

RT1.Balpha^{l} - top
RT1.Balpha^{sd} - middle
DQA1*0101 - bottom

FIG. 1B

```
 40        50        60        70        80        90
 .         .         .         .         .         .
LDKKETIWRIPEFGQLTSFDPQGGLQNIAIIKHNLEILMKRSNSTQAVNKVPEATVF
LDKKETIWRIPEFGQLISFDPQGALRNIAIIKHNLEILMKRSNSTPAVNEVPEATVF
LERKETAWRWPEFSKFGGFDPQGALRNMAVAKHNLNIMIKRYNSTAATNEVPEVTVF
```

```
                                     1       10        20        30
MPLSRALILGVLALTTMLSPCGGQDDIEADHVAAYGINMYQYYESRGQFTHEFDGDEEFYVD
                                          ---GS---TV---H--K--Y--------R----
                                     E--V-----SC-V-L--F-GPS--Y------------
```

peptides synthesized:

RT1.Balpha^{l} - top
RT1.Balpha^{sd} - middle
DQA1*0101 - bottom

FIG. 2A

```
40        50        60        70        80        90
LDKKETIWRIPEFGQLTSFDPQGGLQNIAIIKHNLEILMKRSNSTQAVNKVPEATVF
----------------I------A-R-----------------P----------
-ER---A--W---SKFGG-----A-R-M-VA----N-MI--Y---A-T-E---V---
```

DQA*0101 (62-77) = ALRNMAVAKHNLNIMI

DQB*0501 (61-77) = NSQKEVLEGARASVDR Control

FIG. 2B

```
            1        10        20        30        40
            .        .         .         .         .
DQA1*0101   EDIVADHVASCGVNLYQFYGPSGQYTHEFDGDEEFYVDLERKE
DQA1*0102   ---------------------------------Q---------
DQA1*0103   ------------------------F--------Q------K--
DQA1*0104   -G-----------------------------------------
DQA1*0201   ----------Y------S------F------------------
DQA1*03011  ----------Y------S-------S-----------------
DQA1*03012  ----------Y------S-------S-----------------
DQA1*0302   ****------Y------S-------S-----------------
DQA1*0401   ----------Y------S---------------Q-----G---
DQA1*05011  ----------Y------S---------------Q-----G---
DQA1*05012  ----------Y------S---------------Q-----G---
DQA1*05013  ************-----S---------------Q-----G---
DQA1*0601   *****************S------F--------Q-----G---

                  110       120       130       140
                  .         .         .         .
DQA1*0101   GQPNTLICLVDNIFPPVVNITWLSNGQSVTEGVSETSFLSKSD
DQA1*0102   -------------------------------D-----------
DQA1*0103   *******************************************
DQA1*0104   *******************************************
DQA1*0201   --------------------------H----------------
DQA1*03011  --------------------------H----------------
DQA1*03012  *******************************************
DQA1*0302   --------------------------H----------------
DQA1*0401   --------------------------H----------------
DQA1*05011  ----I---------------------H----------------
DQA1*05012  *******************************************
DQA1*05013  ----I---------------------H----------------
DQA1*0601   *******************************************
```

A — A

FIG. 3A

FIG. 3B

```
A
      50        60        70        80        90        100
      .         .         .         .         .         .
TAWRWPEFSKFGGFDPQGALRNMAVAKHNLNIMIKRYNSTAATNEVPEVTVFSKSPVTL
-----------------------------------------------------------
-------------------------------------------****************
-------------------------------------------****************
-V-KL-L-HRL R----F--T-I--L------L---S----------------------
-V-QL-L-RR-RR----F--T-I--L------V---S----------------------
-V-QL-L-RR-RR----F--T-I--L------V---S-------***************
-V-QL-L-RR-RR----F--T-I--L------V---S----------------------
-V-CL-VLRQ- R----F--T-I--T------L---S----------------------
-V-CL-VLRQ- R----F--T-I--L-----SL---S----------------------
-V-CL-VLRQ- R----F--T-I--L-----SL---S-------***************
-V-CL-VLRQ- R----F--T-I--L-----SL---S----------------------
-V-CL-VLRO- R----F--T-I--T------L---S**********************

      150       160       170       180       190       200
      .         .         .         .         .         .
SFFKISYLTFLPSADEIYDCKVEHWGLDQPLLKHWEPEIPAPMSELTETVVCALGLSV
----------------------------------------------------------
**********************************************************
**********************************************************
----------------------------E-----------------------------
----------------------------E-----------T-----------------
**********************************************************
--------------D-------------E-----------T-----------------
----------------------------E-----------------------------
---------L----E-S-----------K-----------------------------
**********************************************************
A
---------L----E-S-----------K-----------------------------
**********************************************************
B                                                          B
```

SUBSTITUTE SHEET (RULE 26)

FIG. 3C

B ——— B

```
                 210       220       230
                   .         .         .
DQA1*0101   GLVGIVVGTVFIIQGLRSVGASRHQGPL
DQA1*0102   --M-------------------------
DQA1*0103   ****************************
DQA1*0104   ****************************
DQA1*0201   ----------L--R--------------
DQA1*03011  ----------L--R--------------
DQA1*03012  ****************************
DQA1*0302   ----------L- R--------------
DQA1*0401   -------------R--------------
DQA1*05011  -------------R--------------
DQA1*05012  ****************************
DQA1*05013  -------------R--------------
DQA1*0601   ****************************
```

FIG. 4A

```
               1        10        20        30        40
DQB1*0501      RDSPEDFVYQFKGLCYFTNGTERVRGVTRHIYNREEYVRFDSD
DQB1*0502      -------------------------------------------
DQB1*05031     -------------------------------------------
DQB1*05032     ********************-----------------------
DQB1*0504      ********---------------------Y-------------
DQB1*06011     --P-----L---AM-----------Y---Y------D------
DQB1*06012     ************AM-----------Y---Y------D------
DQB1*0602      --------F----M-----------L---Y-------A-----
DQB1*0603      -------------M-----------L-----------A-----
DQB1*0604      -------------M-----------L-----------A-----
DQB1*0605      -------------------------L---Y-------A-----
DQB1*0606      ********************-----L---Y-------A-----
DQB1*0607      ********-----M-----------L-----------A-----
DQB1*0608      ********-----M-----------L-----------A-----
DQB1*0201      -------------M-----------L-S-S------I------
DQB1*0301      *****-------AM-----------Y---Y-------A-----
DQB1*0302      -------------M-----------L---Y-------A-----
DQB1*03031     -------------M-----------L---Y-------A-----
DQB1*03032     -------------M-----------L---Y-------A-----
DQB1*0304      ------------AM-----------Y---Y-------A-----
DQB1*0305      ****---------M---------------Y-------A-----
DQB1*0401      --------F----M--------L------Y-------A-----
DQB1*0402      --------F----M---------------Y-------A-----
```

A A

B B

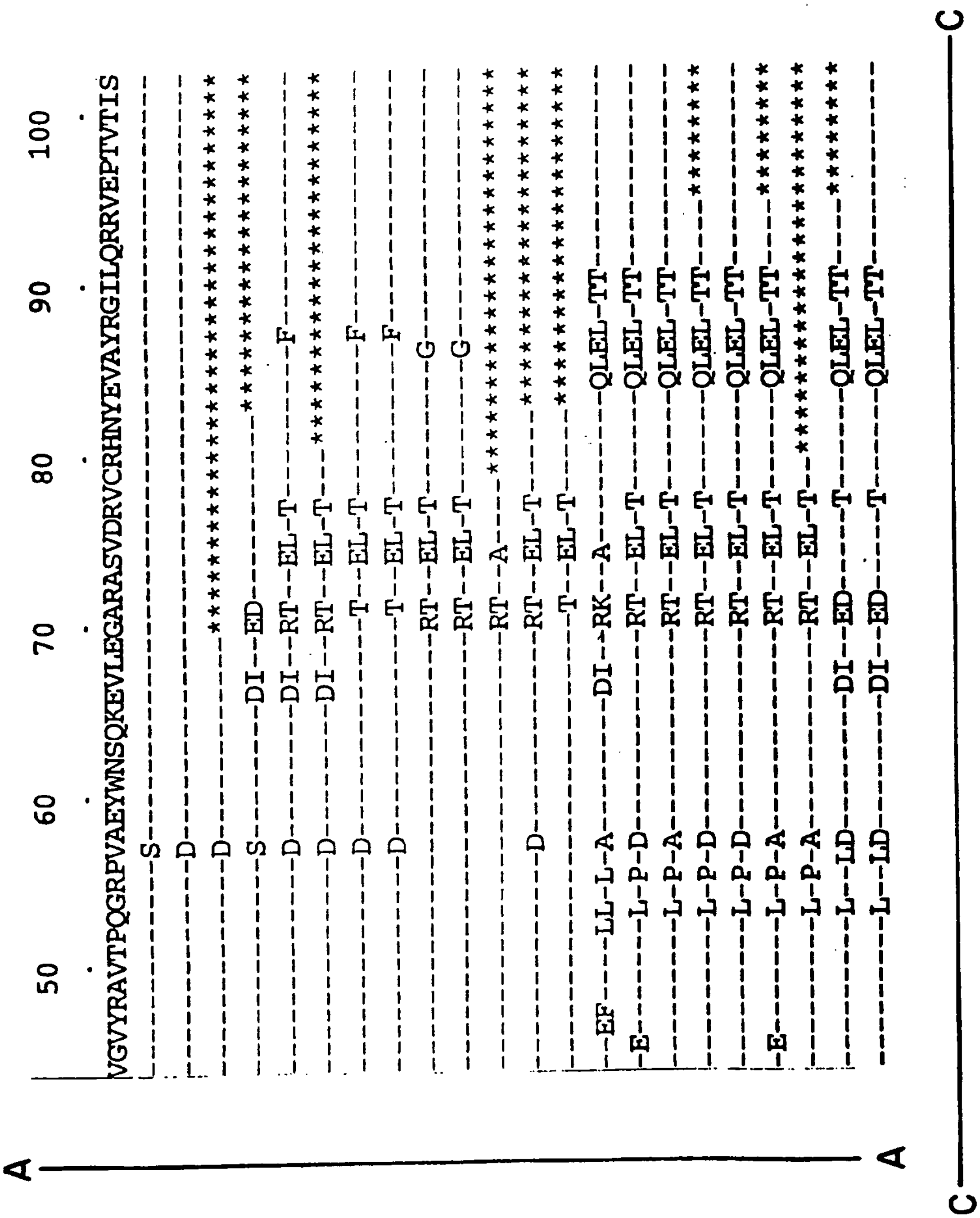

50
60
70
80
90
100
VGVYRAVTPQGRPVAEYWNSQKEVLEGARASVDRVCRHNYEVAYRGILQRRVEPTVTIS
A
A
C
C

FIG. 4B

```
             110       120       130       140
               .         .         .         .
DQB1*0501    PSRTEALNHHNLLICSVTDFYPSQIKVRWFRNDQEETAGVVST
DQB1*0502    -----------------------H-------------------
DQB1*05031   ------*************************************
DQB1*05032   *******************************************
DQB1*0504    *******************************************
DQB1*06011   -------------V--------G--------------------
DQB1*06012   *******************************************
DQB1*0602    -------------V--------G--------------------
DQB1*0603    ------*************************************
DQB1*0604    -------------V--------G----Q---------------
DQB1*0605    ------*************************************
DQB1*0606    *******************************************
DQB1*0607    *******************************************
DQB1*0608    *******************************************
DQB1*0201    -------------V--------A--------------------
DQB1*0301    -------------V--------A--------------T-----
DQB1*0302    -------------V--------A--------------T-----
DQB1*03031   *******************************************
DQB1*03032   -------------V--------A--------------T-----
DQB1*0304    *******************************************
```

B — B

D — D

FIG. 4C

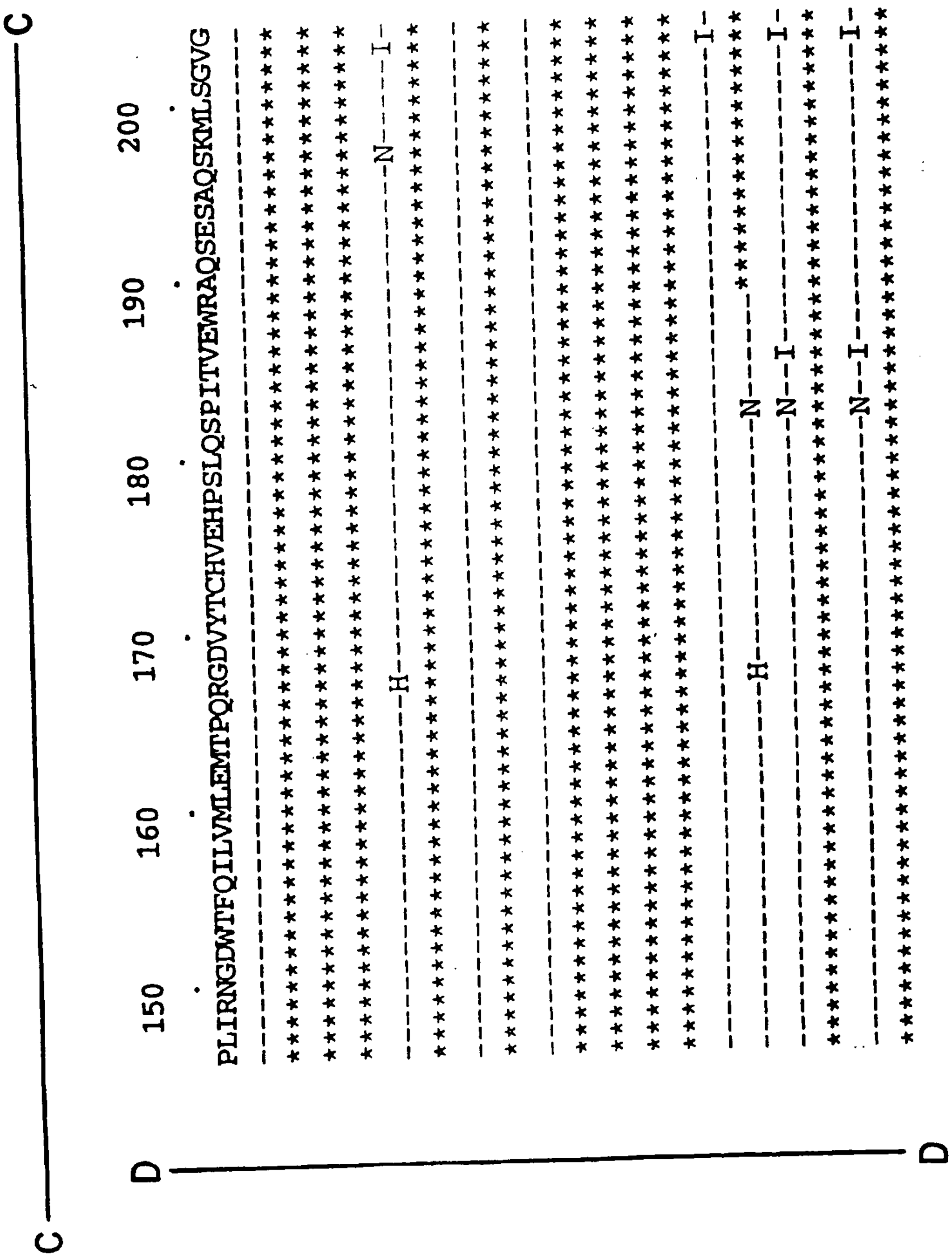

C
C
D
D
150
160
170
180
190
200
PLIRNGDWTFQILVMLEMTPQRGDVYTCHVEHPSLQSPITVEWRAQSESAQSKMLSGVG

FIG. 4D

FIG. 5

IMMUNOMODULATORY PEPTIDES FROM MHC CLASS II ALPHA CHAINS

DQA1*0101	(62-77):	ALRNMAVAKHNLNIMI
RT1.Dα	(61-75):	ANIAVDKANLDIMIK
RT1.Bα	(62-78):	GLQNIAIIKHNLEILMK

CONTROL PEPTIDE

DQB*0501	(62-77):	NSQKEVLEGARASVDR

FIG. 6

Peptide	10µg/ml	5µg/ml	2.5µg/ml	1.25µg/ml	0.625µg/ml	0.312µg/ml	0.156µg/ml	0.078µg/ml
LEW/WF								
DQAlpha#1	107.0%	86.0%	62.3%	55.2%	40.6%	-37.9%	-4.9%	-63.2%
DQAlpha#2	104.6%	96.0%	81.0%	62.7%	32.5%	8.4%	25.3%	1.3%
DQAlpha#3	123.1%	90.2%	66.2%	52.8%	52.1%	-8.5%	-5.0%	-74.2%
LEW / BN								
DQAlpha#1	86.0%	-2.5%	-12.6%	11.5%	42.6%	51.9%	46.0%	106.0%
DQAlpha#2	1.5%	4.0%	-12.5%	9.7%	7.6%	-21.7%	22.1%	-16.2%
DQAlpha#3	19.4%	-36.0%	-43.4%	-6.9%	-35.7%	-41.0%	-37.7%	-12.3%
BN / LEW								
DQAlpha#1	32.4%	29.9%	22.7%	12.6%	2.9%	23.0%	27.9%	-32.2%
DQAlpha#2	19.0%	-2.0%	-2.2%	5.5%	-8.2%	6.1%	5.5%	-39.7%
DQAlpha#3	52.1%	37.9%	33.5%	49.2%	50.4%	21.2%	8.8%	4.0%
BN /WF								
DQAlpha#1	124.9%	122.5%	124.7%	114.5%	108.2%	63.0%	22.8%	16.9%
DQAlpha#2	117.6%	110.8%	117.7%	105.2%	99.7%	26.5%	43.6%	30.7%
DQAlpha#3								
LEW / BN	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	3.12µg/ml	1.56µg/ml	0.78µg/ml
DQAlpha#1	220.8%	214.9%	200.4%	154.6%	88.5%	40.3%	82.8%	18.2%
DQAlpha#2	118.6%	90.1%	88.3%	37.6%	-28.2%	-97.4%	-55.0%	-65.9%
DQAlpha#3	104.1%	103.0%	85.5%	64.6%	21.7%	-11.3%	-5.3%	2.3%
Antigen/MBP	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	3.12µg/ml	1.56µg/ml	0.78µg/ml
DQAlpha#1	131.7%	115.5%	86.5%	86.5%	95.1%	75.3%	38.3%	59.8%
DQAlpha#2	139.3%	97.6%	71.8%	116.3%	108.0%	61.0%	-18.4%	-19.3%
DQAlpha#3	120.6%	56.7%	74.1%	92.4%	89.3%	24.5%	-46.1%	-137.9%

FIG. 7

ConA	10µg/ml	5µg/ml	2.5µg/ml	1.25µg/ml	0.625µg/ml	0.312µg/ml	0.156µg/ml	0.078µg/ml
DQAlpha#1	19.5%	-73.0%	-54.6%	-48.8%	-14.5%	-25.1%	100.6%	100.6%
DQAlpha#2	26.5%	.3%	-36.4%	-12.1%	8.8%	-14.2%	100.6%	100.6%
DQAlpha#3	23.2%	11.3%	20.5%	-27.3%	-16.6%	.1%	100.9%	100.9%
ConA	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	3.12µg/ml	1.56µg/ml	0.78µg/ml
DQAlpha#1	64.3%	42.1%	-6.3%	-7.1%	-9.3%	4.9%	-5.1%	-.6%
DQAlpha#2	58.9%	13.1%	-23.3%	-69.6%	-49.5%	-41.3%	-28.3%	-35.6%
DQAlpha#3	63.8%	13.4%	-12.8%	-28.8%	-21.9%	1.9%	28.6%	30.2%
Human								
DQ Alpha	10µg/ml	5µg/ml	2.5µg/ml	1.25µg/ml	0.625µg/ml	0.312µg/ml	0.156µg/ml	0.078µg/ml
M/C	28.0%	26.0%	13.8%	8.3%	16.1%	3.7%	6.6%	-5.2%
L/C	41.6%	30.5%	29.8%	24.2%	30.1%	21.2%	26.6%	9.0%
J/C	47.4%	46.5%	16.7%	27.9%	19.0%	1.1%	17.6%	23.6%
J/L	31.9%	17.7%	23.0%	13.4%	17.4%	8.6%	24.1%	-7.3%
L/L	25.0%	-8.8%	7.0%	-2.4%	6.4%	-11.0%	-10.0%	-16.9%
T/L	31.2%	20.6%	63.6%	52.4%	46.9%	27.9%	24.8%	7.8%
DQ Alpha	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	3.12µg/ml	1.56µg/ml	0.78µg/ml
E/C	106.3%	96.7%	64.8%	-14.6%	19.5%	24.2%	47.2%	28.0%
H/C	105.6%	94.3%	44.0%	-9.0%	5.7%	22.4%	35.6%	18.0%
M/C	105.5%	91.2%	16.5%	-58.5%	3.7%	21.8%	21.7%	8.7%
J/L	106.0%	91.3%	42.2%	-2.3%	22.1%	26.5%		
M/L	101.9%	73.0%	33.8%	8.8%	35.4%	22.8%	32.1%	23.2%
T/L	103.8%	90.0%	11.0%	-17.7%	-18.2%	1.2%	30.0%	19.7%
							12.3%	1.6%

FIG. 8

Peptide	250µg/ml	200µg/ml	150µg/ml	100µg/ml	50µg/ml	10µg/ml
LEW/WF						
RT1.D A #1	107.5%	98.6%	80.9%	13.4%	4.9%	20.3%
RT1.D A #2	91.3%	68.0%	30.6%	18.9%	-7.7%	27.5%
RT1.D A #3	88.2%	81.1%	72.6%	-39.8%	26.1%	-34.9%
RT1.D A #4	141.0%	137.1%	133.8%	124.0%	88.0%	22.6%
LEW / BN						
RT1.D A #1	53.4%	59.8%	54.2%	40.5%	-.5%	-34.3%
RT1.D A #2	101.9%	94.1%	66.0%	27.7%	119.1%	21.1%
RT1.D A #3	80.9%	88.1%	35.7%	67.5%	-8.4%	-24.8%
BN/WF						
RT1.D A #1	124.5%	122.7%	122.4%	119.8%	107.7%	-5.6%
RT1.D A #2	110.0%	101.8%	116.5%	110.4%	101.7%	42.8%
Antigen/MBP	250µg/ml	200µg/ml	150µg/ml	100µg/ml	50µg/ml	10µg/ml
RT1.D A #1	115.2%	108.7%	112.2%	112.2%	72.1%	18.1%
RT1.D A #2	110.1%	119.6%	103.3%	103.8%	78.0%	20.1%
RT1.D A #3	104.1%	91.1%	94.7%	103.7%	57.1%	-18.8%
ConA	250µg/ml	200µg/ml	150µg/ml	100µg/ml	50µg/ml	10µg/ml
RT1.D A #1	23.2%	11.3%	20.5%	-27.3%	-16.6%	.1%
Human	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	3.12µg/ml
RT1.D A #1						
H/E	8.7%	9.3%	40.5%	41.9%	24.2%	-10.3%

FIG. 9

Peptide	10µg/ml	5µg/ml	2.5µg/ml	1.25µg/ml	0.625µg/ml	0.312µg/ml	0.156µg/ml	0.078µg/ml
LEW/WF								
RT1.B A#1	115.1%	114.4%	111.4%	81.2%	28.8%	-6.5%	-27.9%	-59.2%
RT1.B A#2	110.7%	110.7%	107.3%	101.4%	97.5%	95.1%	-13.1%	-6.9%
RT1.B A#3	115.1%	114.2%	113.8%	111.3%	65.0%	36.5%	-13.2%	9.8%
RT1.B A#4	109.8%	109.9%	108.7%	105.7%	87.5%	85.7%		
LEW / BN								
RT1.B A#1	63.0%	43.1%	17.0%	21.5%	-28.7%	-54.8%		
RT1.B A#2	71.0%	48.9%	20.0%	10.2%	19.5%	4.4%		
BN / WF								
RT1.B A#1	123.6%	127.2%	125.2%	123.8%	117.2%	79.4%	40.5%	50.1%
RT1.B A#2	124.7%	125.3%	124.9%	123.5%	120.4%	84.2%	29.1%	50.6%
	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	3.12µg/ml	1.56µg/ml	0.78µg/ml
LEW / BN								
RT1.B A#1	220.9%	220.3%	204.0%	187.5%	141.2%	86.9%	85.9%	221.3%
RT1.B A#2	119.4%	119.2%	115.4%	93.0%	32.5%	-18.8%	-63.2%	-96.0%
RT1.B A#3	105.9%	105.8%	104.9%	96.7%	64.5%	35.8%	-1.7%	-21.4%
Antigen/MBP	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	3.12µg/ml	1.56µg/ml	0.78µg/ml
RT1.B A#1	93.9%	108.1%	114.1%	114.9%	70.6%	26.1%	13.3%	25.0%
RT1.B A#2	116.3%	117.3%	111.0%	111.2%	105.6%	100.6%	65.1%	.0%
RT1.B A#3	125.6%	121.7%	118.0%	126.4%	112.1%	124.8%	59.4%	27.7%
ConA	46.3%	-3.4%	-48.5%	-42.5%	-28.4%	-41.1%	101.1%	101.1%

FIG. 10

ConA	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	3.12µg/ml	1.56µg/ml	0.78µg/ml
RT1.B A#1	89.0%	90.7%	84.1%	61.1%	18.9%	-7.8%	-2.9%	-13.6%
RT1.B A#2	82.7%	78.7%	68.0%	40.9%	28.8%	-27.8%	-40.2%	-57.7%
RT1.B A#3	95.3%	83.5%						
Human								
RT1.B Alpha	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	3.12µg/ml	1.56µg/ml	0.78µg/ml
T/E	61.6%	28.0%	9.6%	16.7%	24.6%	7.2%		
A/E	39.8%	30.7%	50.3%	70.6%	60.0%	37.5%	11.5%	-8.9%
H/E	26.5%	-20.5%	8.0%	36.7%	56.4%	22.5%	9.7%	-24.5%

FIG. 11

Peptide	10µg/ml	5µg/ml	2.5µg/ml	1.25µg/ml	0.625µg/ml	0.312µg/ml	0.156µg/ml	0.078µg/ml
LEW/WF								
DQ Beta#1	10.6%	19.5%	9.7%	8.0%	19.8%	-38.9%	-48.5%	-5.0%
DQ Beta#2	29.1%	35.5%	38.7%	43.3%	5.2%	-5.3%	3.4%	-11.5%
DQ Beta#3	-62.2%	-23.7%	64.1%	40.2%	37.5%	-11.5%	-35.4%	-78.1%
LEW / BN								
DQ Beta#1	-23.4%	12.4%	-10.0%	14.7%	32.1%	61.2%	106.0%	106.0%
DQ Beta#2	-1.2%	13.2%	15.8%	4.3%	31.6%	8.9%	-7.0%	113.0%
DQ Beta#3	-78.2%	-28.4%	37.8%	14.1%	35.2%	6.1%	107.0%	107.0%
BN / LEW								
DQ Beta#1	-53.7%	-44.8%	20.6%	-1.5%	-25.6%	-32.2%	-45.4%	-1.8%
DQ Beta#2	30.1%	8.7%	37.3%	2.9%	21.6%	-12.2%	.3%	31.8%
DQ Beta#3	-1.8%	11.5%	11.0%	39.6%	50.3%	38.9%	37.0%	1.3%
	250µg/ml	200µg/ml	150µg/ml	100µg/ml	50µg/ml	10µg/ml		
BN/WF								
DQ Beta#1	-28.0%	35.6%	-17.5%	43.9%	-.9%	8.7%		
DQ Beta#2	-24.5%	36.8%	-62.8%	-20.0%	12.8%	2.8%		
	250µg/ml	200µg/ml	150µg/ml	100µg/ml	50µg/ml	10µg/ml		
LEW / BN								
DQ Beta#1	12.4%	24.3%	2.8%	1.6%	14.7%	46.5%		
DQ Beta#2	2.7%	-8.1%	7.8%	-1.3%	-6.9%	14.3%		
DQ Beta#3	2.7%	-8.1%	7.8%	-1.3%	-6.9%	14.3%		
Antigen/MBP	250µg/ml	200µg/ml	150µg/ml	100µg/ml	50µg/ml	10µg/ml		
DQ Beta#1	-21.2%	-28.3%	-26.1%	-46.3%	2.1%	49.6%		
DQ Beta#2	7.2%	-23.2%	-13.5%	-56.1%	-41.5%	41.6%		
DQ Beta#3	-8.2%	-68.1%	-80.0%	24.6%	-103.3%	-77.1%		

FIG. 12

Human								
DQ Beta	10μg/ml	5μg/ml	2.5μg/ml	1.25μg/ml	0.625μg/ml	0.312μg/ml	0.156μg/ml	0.078μg/ml
M/C	16.8%	2.5%	9.8%	10.4%	-11.3%	3.0%	5.7%	.8%
L_I/C	23.9%	22.3%	21.3%	21.0%	26.3%	27.4%	15.4%	15.4%
J/C	24.8%	20.2%	24.0%	16.6%	9.0%	-.7%	-.4%	2.2%
J/L	13.1%	12.7%	18.2%	25.9%	16.7%	30.2%	19.7%	-17.4%
L_I/L	-18.4%	8.0%	3.2%	4.5%	27.4%	-3.9%	2.3%	-10.4%
T/L	47.7%	47.7%	27.9%	11.5%	33.4%	33.4%	48.4%	20.0%
DQ Beta	100μg/ml	50μg/ml	25μg/ml	12.5μg/ml	6.25μg/ml	3.12μg/ml	1.56μg/ml	0.78μg/ml
E/C	50.2%	26.2%	28.2%	22.5%	30.2%	20.4%	13.4%	18.1%
H/C	58.8%	18.3%	5.2%	-3.9%	24.5%	16.8%	9.8%	9.0%
M/C	51.8%	25.4%	27.1%	32.6%	24.3%	.9%	-13.7%	-4.2%
J/L	23.3%	21.6%	23.5%	18.6%	28.1%	26.5%	30.0%	17.4%
M/L	11.3%	19.9%	25.2%	-10.5%	22.0%	21.2%	28.1%	13.9%
T/L	15.1%	4.5%	8.8%	-5.9%	-2.6%	3.0%	-3.3%	5.4%

FIG. 13

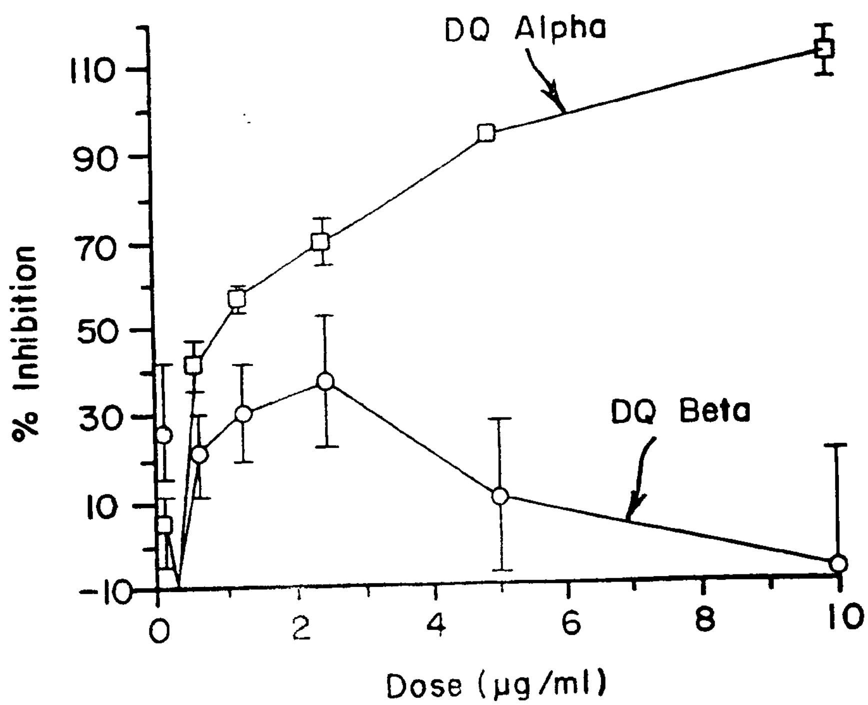

DQ Alpha
DQ Beta
% Inhibition
110
90
70
50
30
10
-10
0
2
4
6
8
10
Dose (μg/ml)

FIG. 14

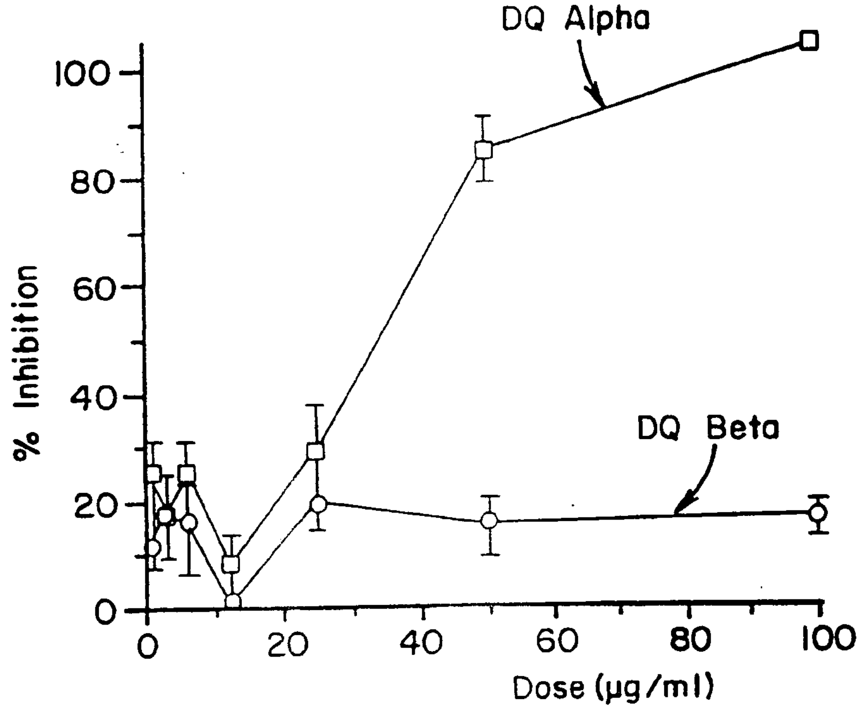

DQ Alpha
DQ Beta
% Inhibition
100
80
60
40
20
0
0
20
40
60
80
100
Dose (µg/ml)

FIG. 15

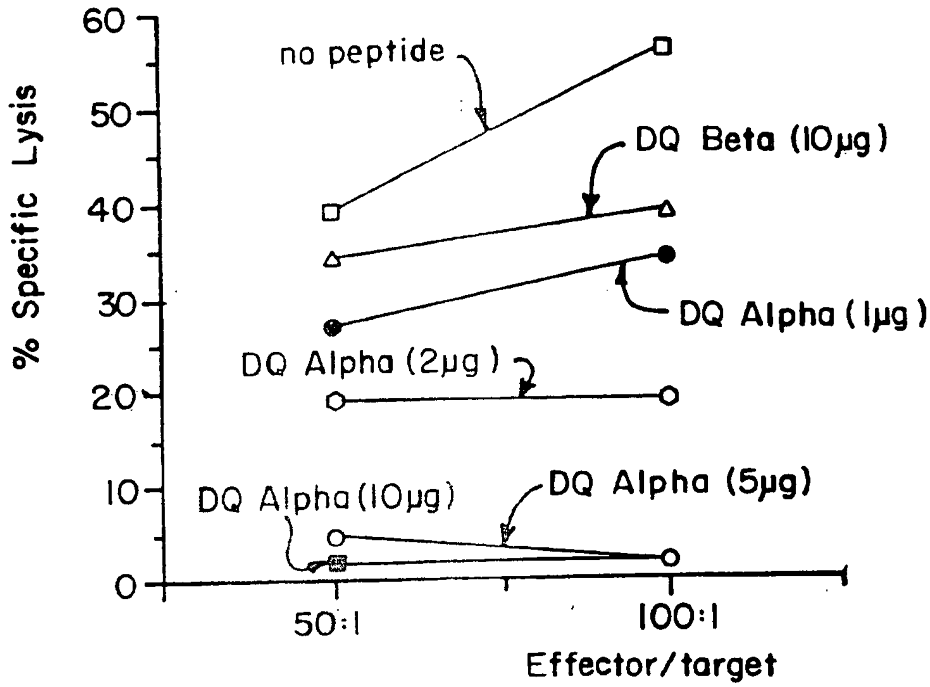

% Specific Lysis
60
50
40
30
20
10
0
no peptide
DQ Beta (10μg)
DQ Alpha (1μg)
DQ Alpha (2μg)
DQ Alpha (10μg)
DQ Alpha (5μg)
50:1
100:1
Effector/target

FIG. 16

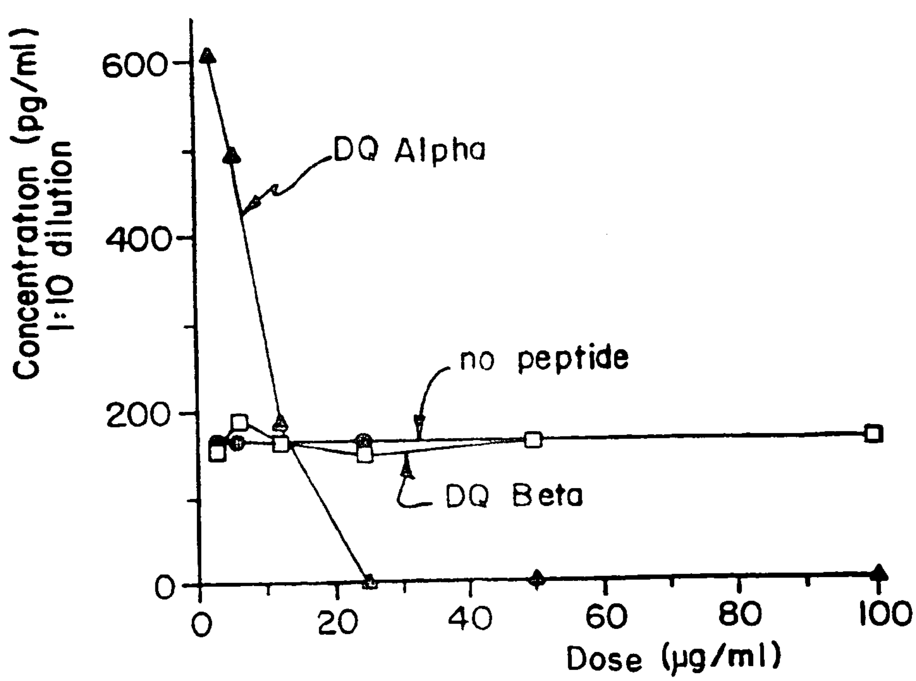
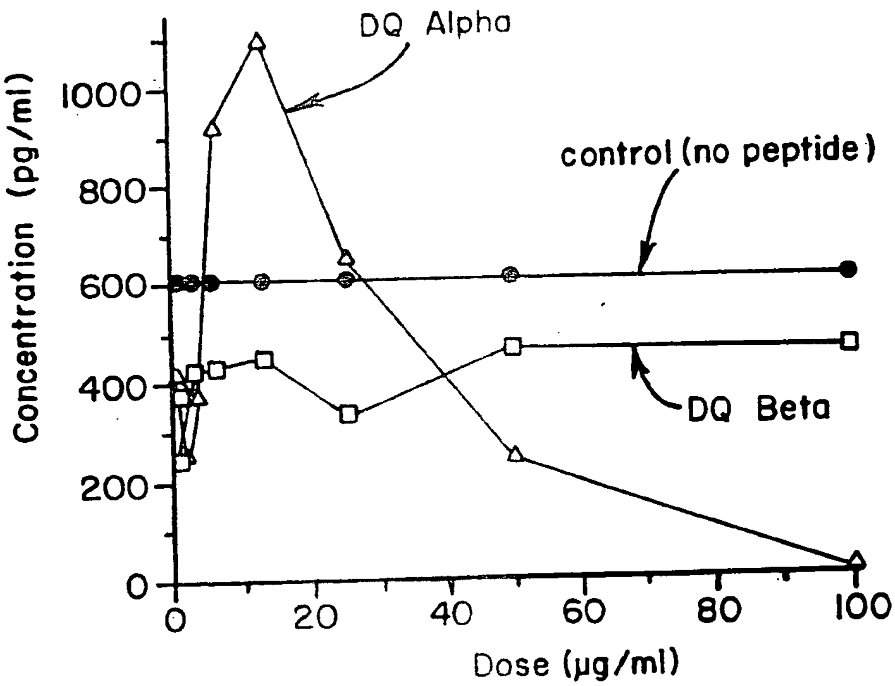

Concentration (pg/ml) 1:10 dilution
600
400
200
0
DQ Alpha
no peptide
DQ Beta
0
20
40
60
80
100
Dose (μg/ml)

FIG. 17


DQ Alpha
control (no peptide)
DQ Beta
Concentration (pg/ml)
0
200
400
600
800
1000
Dose (µg/ml)
0
20
40
60
80
100

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/15662

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/185.1; 514/2, 12, 13, 14, 15, 18; 530/300, 324, 326, 327, 328, 329, 330

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, MEDLINE, EMBASE, WPID
SEARCH TERMS: MHC, ALPHA, SUPPRESS, AUTOIMMUNE, ALLOGRAFT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AGRAWAL et al. T cells that recognize peptide sequences of self MHC class II molecules exist in syngeneic mice. The Journal of Immunology. 15 July 1991, Vol. 147, No. 2, pages 383-390, see entire document,	1-4, 6-10, 14-24
Y	BENICHOU et al. Immunogenicity and tolerogenicity of self-major histocompatibility complex peptides. Journal of Experimental Medicine. November 1990, Vol. 172, pages 1341-1346, see entire document.	1-4, 6-10, 14-24

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 OCTOBER 1996	19 NOV 1996

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	PATRICK NOLAN
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HURTENBACH et al. Prevention of autoimmune diabetes in non-obese diabetic mice by treatment with a class II major histocompatibility complex-blocking peptide. Journal of Experimental Medicine. May 1993, Vol. 177, pages 1499-1504, see entire document.	1-4, 6-10, 14-24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/15662

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 5, 11, 12 and 13
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

A computer readable form was not submitted. Therefore claims 5, 11, 12 and 13 were not searchable.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/15662

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A01N 37/18; A61K 38/00, 38/04, 39/00; C07K 2/00, 4/00, 5/00, 7/00, 14/00, 16/00, 17/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/185.1; 514/2, 12, 13, 14, 15, 18; 530/300, 324, 326, 327, 328, 329, 330